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Association of Codon 108/158 Catechol-O-Methyltransferase Gene Polymorphism With the Psychiatric Manifestations of Velo-Cardio-Facial Syndrome

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Velo-cardio-facial-syndrome (VCFS) is a common congenital disorder associated with typical facial appearance, cleft palate, cardiac defects, and learning disabilities. The majority of patients have an interstitial deletion on chromosome 22q11. In addition to physical abnormalities, a variety of psychiatric illnesses have been reported in patients with VCFS, including schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder. The psychiatric manifestations of VCFS could be due to haploinsufficiency of a gene(s) within 22q11. One candidate that has been mapped to this region is catechol-O-methyltransferase (*COMT*). We recently identified a polymorphism in the *COMT* gene that leads to a valine→methionine substitution at amino acid 158 of the membrane-bound form of the enzyme. Homozygosity for *COMT*158^{met} leads to a 3–4-fold reduction in enzymatic activity, compared with homozygotes for *COMT*158^{val}. We now report that in a population of patients with VCFS, there is an apparent association between the low-activity allele, *COMT*158^{met}, and the development of bipolar spectrum disorder, and in particular, a rapid-cycling form.

KEY WORDS: bipolar disorder, rapid cycling, schizophrenia, catecholamine O-methyltransferase, velo-cardio-facial syndrome

INTRODUCTION

Although a number of Mendelian traits have been mapped by linkage analysis, it has been difficult to map vulnerability genes for complex psychiatric conditions such as bipolar affective disorder (BPD) and schizophrenia. One way to learn more about the underlying molecular and genetic basis of psychiatric illnesses, and perhaps identify potential candidate genes for analysis, is to characterize chromosomal alterations in conditions that are associated with psychiatric manifestations. A good example is velo-cardio-facial syndrome (VCFS), a congenital disorder that results in typical facial appearance, cleft palate, cardiac defects, and learning disabilities [Shprintzen et al., 1978; Scambler et al., 1992; Halford et al., 1993; Morrow et al., 1995]. Approximately 80–85% of patients with VCFS have an interstitial deletion on chromosome 22q11 that is at least 1–2 megabases [Scambler et al., 1992; Halford et al., 1993; Lindsay et al., 1995; Morrow et al., 1995].

In addition to physical anomalies, a variety of psychiatric illnesses have been reported in patients with VCFS, including schizophrenia, attention deficit hyperactivity disorder (ADHD), separation anxiety disorder, obsessive-compulsive disorder (OCD), and BPD [Shprintzen et al., 1992; Goldberg et al., 1993; Chow et al., 1994; Pulver et al., 1994; Papolos et al., 1995]. In a recent evaluation, Papolos et al., [1995] studied 20 children and early adolescents with VCFS and found an unexpectedly high percentage (70%) with bipolar spectrum conditions.

It is very likely that the psychiatric manifestations of VCFS result from the deletion of one or more genes on 22q11. One potential candidate gene found in this re-

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gion is catechol-O-methyltransferase (*COMT*, EC 2.1.1.6) [Scambler et al., 1992; Grossman et al., 1992]. *COMT* inactivates catecholamines and catechol-containing drugs by catalyzing S-adenosyl-L-methionine-dependent methyl conjugation [Axelrod and Tomchick, 1958]. Because of its role in catecholamine metabolism, *COMT* enzymatic activity has previously been measured in the peripheral blood of patients with mood disorders and schizophrenia. However, the results of these studies have been equivocal [Dunner et al., 1977; Philippu et al., 1981; Puzynski et al., 1983; Karege et al., 1987].

COMT enzymatic activity is subject to substantial variability in humans. Approximately 20–25% of Caucasians have been found to express a heat-labile, low-activity enzyme, whereas a somewhat higher percentage express a high-activity enzyme that is heat-stable [Weinshilboum and Raymond, 1977; Scanlon et al., 1979; Spielman and Weinshilboum, 1981; Boudikova et al., 1990; Aksoy et al., 1993]. We and others have recently established that low activity and heat lability are primarily due to a G→A transition at codon 158 of the gene encoding the membrane-bound form of the enzyme (*MB-COMT*), which corresponds to codon 108 of the soluble form (*S-COMT*), leading to a valine→methionine substitution [Lotta et al., 1995; Lachman et al., 1996].

Since dopamine has been postulated to play a role in the pathogenesis of BPD, schizophrenia, and ADHD [Diehl and Gershon, 1992; Kahn and Davis, 1995; Cook et al., 1995], and since dopamine is inactivated by *COMT*, we reasoned that hemizygosity for the low-activity allele, *COMT*158^{met}, could be a factor in the emergence of psychiatric symptoms in VCFS.

MATERIALS AND METHODS

Patient Ascertainment

Patients with VCFS presented with typical mild facial dysmorphology, cleft palate, cardiac defects, and learning disabilities. The ethnic makeup of the patients included 18 Caucasians of mixed North American heritage, 5 European Caucasians, and 3 Hispanics. Microdeletions were detected by FISH analysis and by the loss of heterozygosity for informative 22q11 dinucleotide markers, as previously described [Lindsay et al., 1995; Morrow et al., 1995]. The Diagnostic Interview for Children and Adolescents-Parents and Child Versions-Revised (DICA-R, DICA-RC) [Herjanic and Campbell, 1979; Herjanic and Reich, 1982; Reich et al.,1995] were used to elicit symptoms of childhood psychiatric disorders. Patients above age 18 were administered a computerized version of the Standardized Clinical Diagnostic Interview (SCID) for DSM-III-R. For the adult patients, when possible, a parent DICA-R was also obtained. A semistructured clinical interview with patient and parents was administered by a research psychiatrist to validate and elucidate the specific symptoms reported by the DICA-R and SCID interviews. Two research psychiatrists used these data, as well as a review of all available medical and psychiatric records, to establish a DSM-III-R diagnosis. If a consensus diagnosis could not be made, a meeting was held where the case was discussed and a consensus was reached. Only one patient (AP1079) was not seen by us. She was diagnosed on the basis of a semistructured interview conducted with a parent and a review of the clinical history with her psychiatrist.

The controls used in this study were Caucasian North Americans (46 males, 43 females) with no personal or family history of bipolar disorder or schizophrenia.

Codon 108/158 Polymorphism

Genotype was determined, blind to psychiatric diagnosis, by PCR-RFLP analysis, as described in detail in Lachman et al. [1996]. Briefly, a 210-base pair PCR product was generated using the primers 5′-CTCATCACCATCGAGATCAA and 5′-GATGACCCTGGTGATAGTGG (nucleotides 1881–1900 and 2071–2090, GenBank accession number z26491 [Bertocci et al., 1991; Lundstrom et al., 1991; Tenhunen et al, 1994]). The PCR product (10 μl) was treated with 5 units of *Nla* III for 3 hr at 37°C and separated by electrophoresis through an 8% nondenaturing acrylamide gel.

Statistical analysis was performed using an Instat (GraphPad Software, San Diego, CA) statistical program. A one-tailed Fisher's exact test was applied.

RESULTS

An association study was carried out to determine the frequency of *COMT*158^{met} in psychiatrically affected VCFS patients. The population studied is an extension of the group previously ascertained for psychiatric illness by molecular genetic analysis [Morrow et al., 1995; Papolos et al., 1995]. The single African American individual (AP1088) from the original study was omitted in this association study because substantial racial differences in the frequency of *COMT*158^{met} have been detected (Lachman, unpublished observations). Of the 25 patients with VCFS in this study, 23 had detectable deletions within 22q11 [Lindsay et al., 1995; Morrow et al., 1995]. Two patients (BM102 and BM26) have the typical stigmata of VCFS, but a large 22q11 deletion could not be detected. At least one DSM-III-R diagnosis was found in 23/25 patients (Table I). The high incidence of psychiatric illness in this group, compared with that previously reported in VCFS, may be due in part to ascertainment bias, since a few patients previously diagnosed with psychiatric illness were asked to participate in the study. In addition, patients with psychiatric symptoms may have been more willing to cooperate and undergo a rigorous psychiatric diagnostic interview. There were 17 patients with bipolar spectrum disorder (BPI, BPII, cyclothymia, schizoaffective-manic subype), 11 with attention deficit disorder (ADD) or ADHD, 5 of whom were comorbid for either BPD or cyclothymia, and 2 with OCD. Interestingly, 7 patients with BPD were found to have a rapid-cycling variant. Although this is defined by DSM-III-R as four or more cycles a year, all of the rapidly-cycling patients in this group experienced more frequent episodes, approximately 8–10 a year.

The observed frequency of *COMT*158^{met} was compared with the expected frequency based on 174 chromosomes from Caucasian controls (*COMT*158^{met} = 0.402 and

TABLE I. Psychiatric Diagnosis and *COMT* 108/158 Polymorphism*

Patient no.	Age/sex	Clinical diagnosis	Polymorphism
BM72	8/F	ADHD, avoidance disorder	Val
BM214	17/M	ADD, dysthymia	Val
BM11	11/M	ADD	Met
BM26	8/F	ADD	Met/Met
BM270	18/M	ADD, past major depression	Val
BM139	16/F	ADHD, cyclothymia	Met
BM58	13/M	ADHD, cyclothymia	Met
BM210	12/F	BPII, ADHD	Val
BP90	14/M	BPII (rapid cycling), ADHD, OCD	Met
BM102	17/F	BPII (rapid cycling), ADHD	Met/Met
VCF1	11/F	BPII (rapid cycling)	Met
BM171	25/F	BPII (rapid cycling)	Met
BM128	15/F	BPII (rapid cycling)	Met
BM78	17/M	BPI (rapid cycling)	Met
AP1018	22/M	BPI (rapid cycling), OCD	Met
BM148	12/F	BPII	Met
BM69	15/F	BPII	Met
AP1136	16/M	BPII, ODD	Val
BM122	20/M	BPI	Val
AP1035	22/F	BPII	Val
AP1012	34/M	Schizoaffective-manic, SP	Val
AP1079	29/F	Schizoaffective-manic	Met
BM17	17/M	Dysthymia, ADD	Val

*BPI, bipolar I disorder; BPII, bipolar II disorder; ADHD, attention deficit hyperactivity disorder; ADD, attention deficit disorder without hyperactivity; OCD, obsessive-compulsive disorder; ODD, oppositional defiant disorder; SP, simple phobia.

$COMT158^{val}$ = 0.598, Table II). This is similar to the value established in an analysis of enzyme activity conducted on 900 red blood cell samples [Spielman and Weinshilboum, 1981]. Since association studies can be significantly affected by population differences in allele frequencies, we also genotyped the available parents of the psychiatrically affected VCFS patients (N = 21 individuals, 9 sets of 2 parents, 3 single parents). This analysis actually revealed a somewhat lower frequency of $COMT158^{met}$ (0.357) than that found in controls (Table II). However, since more than half of the parents were unavailable for analysis, we chose the more conservative estimate of 0.402 for this association study.

As shown in Table III, the frequency of $COMT158^{met}$ is increased, ranging between 0.64–1.00 under the different diagnostic categories. Statistical significance was just reached in subgroups of bipolar spectrum disorder that included cyclothymia in the diagnostic scheme. However, the most significant finding occurred in the subgroup of patients who were diagnosed with rapid-cycling BPD. All eight *COMT* alleles, derived from 7 individuals with 22q11 deletions and one with nondeletion VCFS, were $COMT158^{met}$ (P = 0.013). This association is not due to an increase in $COMT158^{met}$ occurring in VCFS. An analysis of 40 chromosomes from 37 anonymous VCFS patients not known to have psychiatric illness (34 with 22q11 deletion, 3 nondeletion) revealed a frequency of 0.425 (data not shown).

DISCUSSION

The primary psychiatric diagnoses found in the population of patients with VCFS described in this report were BPD and ADHD. The increased frequency of mood disorder contrasts with a report showing a relatively high incidence of schizophrenia in VCFS [Pulver et al., 1994]. However, some patients originally diagnosed with schizophrenia (AP1012 and AP1035, patients 1 and 11, respectively, in Pulver et al. [1994]) were found to have a form of BPD when subjected to our diagnostic testing. A consensus diagnosis of schizoaffective disorder was made by both groups for patient AP1079 (patient 14 in Pulver et al. [1994]). The discrepancies may be due to the inherent difficulties in establishing a psychiatric diagnosis in adolescents, especially in the learning-disabled population. However, the finding that these patients responded to mood stabilizers, such as valproate and lithium, is more consistent with the diagnosis of a mood disorder (Papolos, unpublished observations).

A significant number of VCFS patients diagnosed with bipolar spectrum conditions are hemizygous for $COMT158^{met}$, the low activity allele. The strongest as-

TABLE II. Frequency of Codon 108/158 Polymorphism in Controls

	Allele frequency		Genotypes		
Population (no. of chromosomes)	158^{val}	158^{met}	Met/Met	Met/Val	Val/Val
Controls (174)	0.598	0.402	13	44	30
VCFS parents (42)	0.643	0.357	2	11	8

TABLE III. Frequency of Codon 108/158 Polymorphism in VCFS Patients With Psychiatric Illness†

		Met	Val
All diagnoses	Observed	16	9
$P = 0.078$	Expected	10	15
ADHD, ADD	Observed	8	5
$P = 0.22$	Expected	5	8
BPI, BPII	Observed	10	4
$P = 0.13$	Expected	6	9
BPI, BPII, SAM	Observed	11	5
$P = 0.08$	Expected	6	10
BPI, BPII, SAM, CY	Observed	13	5
$P = 0.046$*	Expected	7	11
BPI, BPII, CY	Observed	12	4
$P = 0.035$*	Expected	6	10
Rapid cycling BPD	Observed	8	0
$P = 0.013$*	Expected	3	5

† Allele frequencies under different diagnostic schemes. BPI, bipolar disorder with mania; BPII, with hypomania; CY, cyclothymia; SAM, schizoaffective disorder, manic subtype; ADHD, ADD, attention deficit disorder with and without hyperactivity. Statistical significance was determined by one-tailed Fisher's exact test.
* $P < 0.05$.

sociation was found in patients diagnosed with rapid-cycling BPD. Every *COMT* allele found in this subgroup of patients was *COMT*158^{met}. This suggests that in some patients with VCFS, an increase in catecholamine neurotransmission, caused by low *COMT* activity, can induce an unstable form of BPD. This hypothesis is consistent with observations made in BPD occurring in the general population, in which a rapid-cycling course may develop following treatment with tricyclic antidepressants and monoamine oxidase inhibitors, both of which increase transsynaptic catecholamines [Altshuler et al., 1995]. In this context, it is interesting to note that 2 patients (VCF-1, BM78) in this study appeared to develop rapid-cycling BPD within days of being treated with methylphenidate, a drug that inhibits the dopamine transporter. Methylphenidate had been prescribed after the patients were diagnosed with ADHD by their private physicians. This diagnosis was not supported using our rigorous diagnostic criteria. Rapid cycling persisted after methylphenidate treatment was discontinued.

The results of this study suggest that an analysis of *COMT*158^{met} in BPD occurring in the general population, and in particular, in those who develop a rapid-cycling course, would be of interest. However, so far, we have not detected an association in 60 patients with BPD (unpublished observations). Populations of bipolar patients selected for rapid cycling or other subgroups have not yet been analyzed.

If confirmed, the association of the *COMT* codon 108/158 polymorphism with bipolar spectrum conditions and rapid-cycling BPD in VCFS would be the first description of a specific genetic variant associated with a mood disorder. However, a more extensive analysis and replication in an independent set of patients will be required.

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